PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/00, 15/09, 15/12

A1

(11) International Publication Number: **WO 95/29233**

(43) International Publication Date: 2 November 1995 (02.11.95)

(21) International Application Number: PCT/US95/04860

(22) International Filing Date: 19 April 1995 (19.04.95)

(30) Priority Data:
08/230,491 20 April 1994 (20.04.94) US

(71) Applicant: LUDWIG INSTITUTE FOR CANCER RESEARCH [US/US]; 1345 Avenue of the Americas, New York, NY 10105 (US).

(72) Inventors: RETTIG, Wolfgang, J.; Amriswilstrasse 7, D-88400 Biberach (DE). SCANLAN, Matthew, J.; 1275 York Avenue, New York, NY 10021 (US). GARIN-CHESA, Pilar; Amriswilstrasse 7, D-88400 Biberach (DE). OLD, Lloyd, J.; 1345 Avenue of the Americas, New York, NY 10105 (US).

(74) Agent: HANSON, Norman, D.; Felfe & Lynch, 805 Third Avenue, New York, NY 10022 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: ISOLATED NUCLEIC ACID MOLECULE CODING FOR FIBROBLAST ACTIVATION PROTEIN α AND USES THEREOF

(57) Abstract

The invention describes the identification and isolation of nucleic acid molecules which code for fibroblast activation protein alpha, or "FAPα". Various applications of the isolated molecules are also described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

ISOLATED NUCLEIC ACID MOLECULE CODING FOR FIBROBLAST ACTIVATION PROTEIN α AND USES THEREOF

FIELD OF THE INVENTION

This invention relates to certain molecules associated with cancer cells and reactive with tumor stromal cells. More particularly, it relates to fibroblast activation protein alpha ("FAPα" hereafter). The molecule has previously been identified immunologically, but nucleic acid molecules coding for it had not been isolated or cloned. This, inter alia, is the subject of the invention. The protein has a molecular weight of from about 88 to about 95 kilodaltons as determined by SDS-PAGE. This molecule is characterized by a number of features and properties which are shared by and characteristic of membrane bound enzymes, suggesting very strongly that it, too, is a membrane bound enzyme. The nucleic acid molecules, which are a key part of the invention, are useful both as probes for cell expressing FAPα, and as starting materials for recombinant production of the protein. The recombinant protein can then be used to produce monoclonal antibodies specific for the protein, and are thus useful diagnostic agents themselves.

BACKGROUND AND PRIOR ART

The invasive growth of epithelial cancers is associated with characteristic cellular and molecular changes in the supporting stroma. For example, epithelial cancers induce the formation of tumor blood vessels, the recruitment of reactive tumor stromal fibroblasts, lymphoid and phagocytic infiltrates, the release of peptide mediators and proteolytic enzymes, and the production of an altered extracellular matrix (ECM). See, e.g., Folkman, Adv. Cancer Res. 43: 175-203 (1985); Basset et al., Nature 348: 699-704 (1990); Denekamp et al., Cancer Metastasis Rev. 9: 267-282 (1990); Cullen et al., Cancer Res. 51: 4978-4985 (1991); Dvorak et al., Cancer Cells 3: 77-85 (1991); Liotta et al., Cancer Res. 51: 5054s-5059s (1991); Garin-Chesa et al., J. Histochem. Cytochem. 37: 1767-1776 (1989). A highly consistent molecular trait of the stroma in several common histologic types of epithelial

cancers is induction of the fibroblast activation protein (FAPα), a cell surface glycoprotein with an observed M_r of 95,000 originally discovered with a monoclonal antibody, mAb F19, raised against proliferating cultured fibroblasts. See Rettig et al., Cancer Res. 46: 6406-6412 (1986); Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. USA 87: 7235-7239 (1990); Rettig et al., Cancer Res. 53: 3327-3335 (1993). Each of these four papers is incorporated by reference in its entirety.

Immunohistochemical studies such as those cited supra have shown that FAPα is transiently expressed in certain normal fetal mesenchymal tissues but that normal adult tissues are generally FAPα$^-$. Similarly, malignant epithelial, neural and hematopoietic cells are FAPα$^-$. However, most of the common types of epithelial cancers, including >90% of breast, lung, skin, pancreas, and colorectal carcinomas, contain abundant FAPα$^+$ reactive stromal fibroblasts. Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990). The FAPα$^+$ tumor stromal fibroblasts almost invariably accompany newly-formed tumor blood vessels, forming a distinct cellular compartment interposed between the tumor capillary endothelium and the basal aspect of malignant epithelial cell clusters. While FAPα$^+$ stromal fibroblasts are found in both primary and metastatic carcinomas, benign and premalignant epithelial lesions, such as fibroadenomas of the breast and colorectal adenomas only rarely contain FAPα$^+$ stromal cells. In contrast to the stroma-specific localization of FAPα in epithelial neoplasms, FAPα is expressed in the malignant cells of a large proportion of bone and soft tissue sarcomas. Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988). Finally, FAPα$^+$ fibroblasts have been detected in the granulation tissue of healing wounds (Garin-Chesa et al., supra). Based on the restricted distribution pattern of FAPα in normal tissues and its uniform expression in the supporting stroma of many epithelial cancers, clinical trials with ^{131}I-labeled mAb F19 have been initiated in patients with metastatic colon cancer

(Welt et al., Proc. Am. Assoc. Cancer Res. 33: 319 (1992)) to explore the concept of "tumor stromal targeting" for immunodetection and immunotherapy of epithelial cancers.

The induction of FAPα$^+$ fibroblasts at times and sites of tissue remodeling during fetal development, tissue repair, and carcinogenesis is consistent with a fundamental role for this molecule in normal fibroblast physiology. Thus, it is of interest and value to isolate and to clone nucleic acid molecules which code for this molecule. This is one aspect of the invention, which is described in detail together with other features of the invention, in the disclosure which follows.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows results obtained from immunoprecipitation studies carried out on detergent extracts of Trans ^{35}S-labeled cells. The study was designed to immunoprecipitate FAPα and CD26. Cell types were SW872, which is a human sarcoma cell line, COS-FAP, which is a cell line transfected with a vector coding for FAPα, i.e., pFAP-38, described in the application, and COS-CD26, which is a COS cell line transfected with a CD26 coding plasmid. Extracts were precipitated with anti-FAPα monoclonal antibody F19, anti-CD26 mAb EF-1, or a negative control mouse Ig.

Figure 2A presents Northern blot analysis of FAPα expression in a cell line (ovarian cancer SK-OV6), which has FAPα$^-$/CD26$^+$ phenotype), as well as two cell lines (fibroblasts WI-38 and GM 05389), which have FAPα$^+$/CD26$^+$ phenotype.

Figure 2B shows γ-actin expression for the cell lines of figure 2A.

Figure 3 compares the deduced amino acid sequence for FAPα, and the known sequence of CD26. The alignment has been optimized.

Figure 4 depicts heterodimer formation between FAPα and CD26 in COS-1 transfectants.

Figures 5A-5H, inclusive, display immunohistochemical detection of FAPα and CD26 in various cancers. In figures 5A and 5B, breast cancer is studied, for FAPα (figure 5A), and

CD26 (figure 5B). In figures 5C and 5D, malignant fibrous histiocytoma are studied, for FAPα (figure 5C), and CD26 (figure 5D). Dermal scar tissue is examined in figures 5E (FAPα), and 5F (CD26). Renal cell carcinoma is studied in figure 5G (FAPα), and 5H (CD26).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

Fibroblast cell line WI-38 had been observed, previously, to react with mAb F19 (Rettig et al., Canc. Res. 46: 6406-6412 (1986); Rettig et al., Proc. Natl. Acad. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993)). It was used in the experiments which follow.

A cDNA library was prepared from WI-38, using well known techniques and commercially available materials. Specifically, the library was constructed in expression vector pCDNAI, using the Fast Track mRNA isolation kit, and Librarium cDNA phagemid system. Once the library was prepared, the vectors were electroporated into cell line E. coli MC 1061/P3. The pCDNAI expression vector contains an antibiotic resistance gene, so the E. coli were selected via antibiotic resistance. The colonies which were resistant were then used in further experiments. The plasmid DNA from the colonies was obtained via alkaline lysis and purification on $CsCl_2$, in accordance with Sambrook et al, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab, Cold Spring Harbor, N.Y. 2d Ed. 1989). The technique is well known to the art, but is incorporated by reference herein.

Once the plasmid DNA was isolated, it was used to transfect COS-1 cells, which were then cultured for forty-eight hours, after which these was tested with antibody coated dishes. The mAbs used included F19, as described by Rettig et al., (1986), supra, which is incorporated by reference in its entirety. As COS-1 cells are normally FAPα$^-$, any positive results indicated the presence of the coding sequence. The immunoselection protocol was that of Aruffo et al., Proc. Natl. Acad. Sci USA 84: 3365-3369 (1987), incorporated by

reference herein.

Plasmid DNA from positive clones was recovered, in accordance with Hirt, J. Mol. Biol. 26: 365-369 (1967), reintroduced into E. coli MC 1061/P3, and reselected into COS-1 cells.

The protocol presented herein was followed for four rounds. After this, the plasmid DNA of 50 isolated bacterial colonies was purified, using the Qiagen plasmid kit. Of the colonies, 27 clones were found to contain identical 2.8 kb inserts, as determined by EcoRI restriction enzyme mapping. Several of these were found to contain FAPα-specific cDNA, via transient expression in COS-1 cells and direct immunofluorescence staining. One of these clones, i.e., "pFAP.38" was selected for further study, as elaborated upon infra.

Example 2

Once pFAP.38 had been identified, it was tested together with a vector coding for known cell surface marker CD26 ("pCD26"), as well as with control vector pCDNA I.

In these experiments, COS-1 cells were transfected with one of pFAP.38, pCD26, or pCDNAI. After forty-eight hours, the transfectants were tested, using the well known MHA rosetting assay for cell surface antigen expression. In these experiments, mAb F19, which is FAPα specific, was used, together with mAb EF-1, which is CD26 specific. Also used were four other FAPα specific mAbs, i.e., FB23, FB52, FB58 and C48. Also tested were two cancer cell lines, which are known to react with mAb F19 (SW872 liposarcoma), or EF-1 (SK-OV6 ovarian cancer). The results are set forth in Table 1, which follows.

Table 1. Cell surface expression of multiple FAPα epitopes and CD26 in human cells and COS-1 cell transfectants.

Target Cell	F19	FB23	FB52	FB58	C48	EF-1
Human cells						
SW872 liposarcoma	95%	>95%	>95%	>95%	>95%	-
SW-OV6 ovarian cancer	-	-	-	-	-	>95%
COS-1 transfectants						
COS-pCDNAI control	-	-	-	-	-	-
COS-pFAP 38	40%	30%	40%	20%	20%	-
COS-pCD26	-	-	-	-	-	40%

Example 3

Immunoprecipitation studies were then carried out to identify the antigen being targeted by the antibodies.

Cells were metabolically labelled with Trans ^{35}S-label, (ICN), extracted with lysis buffer (0.01 M Tris-HCl/0.15 M NaCl/0.01 M $MgCl_2$/0.5% Nonidet P-40/aprotinin (20 ug/ml)/2 mM phenylmethyl- sulfonyl fluoride), and then immunoprecipitated. The protocols used are all well known, as will be seen by reference to Rettig et al., Canc. Res. 53: 3327-3335 (1993); and Fellinger et al., Canc. Res. 51: 336-340 (1991), the disclosures of which are all incorporated by reference in their entirety. Precipitating mAbs were negative control mouse Ig, mAb F19, or EF-1. Control tests were carried out with mock transfected COS-1 cells. Following immunoprecipitation, the immunoprecipitates were separated on $NaDOdSO_4$/PAGE, under reducing conditions. In some experiments, an additional test was carried out to determine whether or not the immunoprecipitated material was glycosylated. In these experiments, cell extracts were fractionated with Con A-SEPHAROSE prior to immunoprecipitation. Following immunoprecipitation, but prior to fractionation on $NaDodSO_4$/PAGE, these precipitates were

digested with N-Glycanase.

The results are shown in figure 1. In COS-1 cells, pFAP.38 directs expression of an 88 kd protein species (as determined via SDS-PAGE), which is slightly smaller than the 95 kd FAPα species produced by SW872, or cultured fibroblasts. Digestion with N-Glycanase produced peptides of comparable size (i.e., 74 kd versus 75 kd), showing that the glycosylation of the FAPα protein in COS-1 cells is different than in the human cell lines.

Example 4

Classic Northern blot analysis was then carried out, using the mRNA from FAPα$^+$ fibroblast cell lines WI-38 and GM 05389, and FAPα$^-$ovarian cancer cell line SK-OV6. Using the procedures of Sambrook et al., supra, five micrograms of mRNA from each cell line were tested. The probes used were ^{32}P labelled, and were prepared from a 2.3 kb ECO I fragment of pFAP.38, a 2.4 kb Hind III fragment of CD26, and a 1.8 kb BamHI fragment of γ-actin cDNA. These fragments had been purified from 1% agarose gels.

Figure 2 presents these results. The extracts of FAPα$^+$ fibroblast strains show a 2.8 kb FAP mRNA species, but extracts of SK-OV6 do not. A γ-actin mRNA species (1.8 kb), is seen in all species.

Example 5

The cDNA identified as coding for FAPα was subjected to more detailed analysis, starting with sequencing. The classic Sanger methodology, as set forth in Proc. Natl. Acad. Sci. USA 74: 5463-5467 (1977), was used to sequence both strands of the cDNA. Once this was secured, an amino acid sequence was deduced therefrom. This information is presented in SEQ ID NO: 1. The sequence was then compared to the known amino acid sequence of CD26 (Morimoto et al., J. Immunol. 143: 3430-3437 (1989)). Figure 3 presents the comparison, using optimized sequence alignment. Any gaps in the comparison are indicated by asterisks, while identical amino acids are shown by dashes in the CD26 sequence. A hydrophobic, putative transmembrane sequence is double underlined, while potential N-glycosylation

sites are single underlined.

The sequence analysis shows a 2812 base pair insert, wherein 2277 base pairs constitute the open reading frame. This ORF extends from start codon ATG at nucleotide 209, to stop codon TAA at 2486.

The deduced peptide is 759 amino acids long, and has a molecular weight of 88,233. In contrast, N-Glycanase digested, immunopurified FAPα was reported to have an estimated M_r of 75,000 on NaDodSO$_4$/PAGE (Rettig et al., Canc. Res. 53: 3327-3335 (1993)). A TATA box is found 83 base pairs upstream of the start codon. A polyadenylation signal and a poly-A tail were found in the 5'-untranslated region of the insert.

A GenBank data base search was then carried out. The most closely related genes found were those encoding dipeptidyl peptidase IV homologues (DPPIV; EC 3.4.14.5), with human DPPIV (also known as T-cell activation antigen CD26), showing 61% nucleotide sequence identity, and 48% amino acid sequence identity.

The second set of related genes are human, rat, and bovine homologues of DPPX, a gene of unknown function widely expressed in brain and other normal tissues. The predicted human DPPX gene product shows about 30% amino acid sequence identity with FAPα and CD26. The FAPα molecule exhibits structural features typical of type II integral membrane proteins, including a large COOH-terminal extracellular domain, a hydrophobic transmembrane segment, and a short cytoplasmic tail. The putative extracellular domain contains six potential N-glycosylation sites, 13 cysteine residues (8 of which are conserved between FAPα and CD26), and three segments corresponding to highly conserved catalytic domains characteristic of serine proteases, such as DPPIV. These conserved sequences are presented in Table 2, which follows. Comparisons to DPPIV and DPPX were made via Morimoto et al., supra; Wada et al., Proc. Natl. Acad. Sci. USA 89: 197-201 (1992); Yokotani et al., Human Mol. Genet. 2: 1037-1039 (1993).

Table 2. Putative catalytic domains of FAPα, DPPIV and DPPX.

	625	701	733
Human FAPα	....WGWSYEI.....	GTADDNV.....	DQNHGLS....
Human DPPIV	....WGWSYGG.....	GTADDNV.....	DEDHCIA....
Mouse DPPIV	....WGWSYGG.....	GTADDNV.....	DEDHGIA....
Rat DPPIV	....WGWSYGG.....	GTADDNV.....	DEDHGIA....
Yeast DPPIV	....FGWSYGG.....	GTGDDNV.....	DSDHSIR....
Human DPPX	....FGKDYGG.....	PTADEKI.....	DESHYFT....
Rat DPPX	....FGKDYGG.....	ATADEKI.....	DESHYFH....
Bovine DPPX	....FGKDYGG.....	ATEDEKI.....	DESHYFS....

Example 6

An additional set of experiments were carried out to determine whether FAPα related sequences are present in non-human species. To do so, human, mouse, and Chinese hamster genomic DNA was digested using restriction enzymes, and tested, via Southern blotting, using the 2.3 kb fragment, labelled with ^{32}P, describes supra. Hybridization was carried out using stringent washing conditions (0.1 x SSC, 0.1% NaDodSO$_4$, 68°C). Cross-hybridization was readily observed with both the mouse and hamster DNA, suggesting the existence of highly conserved FAPα homologues. In control experiments using the CD26 cDNA fragment described supra, no evidence of cross hybridization was observed.

Example 7

The CD26 molecule shares a number of biochemical and serological properties with FAPβ, which is a previously described, FAPα associated molecule having a molecular weight of 105 kd, and found on cultured fibroblasts and melanocytes (Rettig et al., Canc. Res. 53: 3327-3335 (1993)). Cotransfection experiments were carried out to determine whether FAPβ is a CD26 gene product. To test this, the same protocols were used which were used for transfection with pFAP.38 or pCD26, as described supra, but using the two

vectors. The results presented supra showed that cotransfection efficiency was about 40% for each vector, so about 10-20% of cell should be cotransfected.

Following cotransfection, the COS-1 cells were Trans ^{35}S-labeled, as described supra, then lysed, also as described supra.

The resulting cell extracts were separated on Con A SEPHAROSE, and the antigen (FAPα and/or CD26) were recovered in the Con A-bound fraction. The bound fraction was eluted with 0.25 M α-D-mannopyranoside. Immunoprecipitation was then carried out, as described supra, and the precipitates were separated on $NaDodSO_4$/PAGE, also as discussed supra.

Figure 4 shows these results, together with results from single transfection experiments. Those cells transfected only with pFAP.38 produce FAPα, but not FAPß (determined from mAb F19 immunoprecipitates). They also produce no CD26 antigen (tested with EF-1). Those cells transfected with pCD26 alone produce CD26 but no FAPα. Cotransfectants produce CD26 and FABα/FAPß heteromers, as determined in the mAb F19 precipitates. This result provides direct evidence that FAPß is a CD26 gene product.

Example 8

It has been observed previously that some cultured human cell types coexpress FAPα and CD26, and show FAPα/CD26 heteromer formation. In vivo distribution patterns of FAPα and CD26, however, as determined in previous immunohistochemical studies, appeared to be non-overlapping. (See Rettig et al., Proc. Natl. Acad. Sci. USA 85: 3110-3114 (1988); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7329 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993); Stein et al., in Knapp et al., eds. Leukocyte typing IV-white cell differentiation antigens, pp 412-415 (Oxford University Press, N.Y. 1989), pp. 412-415; Möbious et al., J. Exp. Immunol. 74: 431-437 (1988)). In view of the potential significance of FAPα/CD26 coassociation, tissue distribution was reexamined, via side by side immunohistochemical staining of normal tissues and lesional tissues known to contain FAPα$^+$

fibroblasts or FAPα+ malignant cells.

To test the samples, they were embedded in OCT compound, frozen in isopentane precooled in liquid nitrogen, and stored at -70°C until used. Five micrometer thick sections were cut, mounted on poly-L-lysine coated slides, air dried, and fixed in cold acetone (4°C, for 10 minutes). The sections were then tested with mAbs (10-20 ug/ml), using the well known avidin-biotin immmuno-peroxidase method, as described by, e.g., Garin-Chesa et al., J. Histochem. Cytochem. 37: 1767-1776 (1989); Garin-Chesa et al., Proc. Natl. Acad. Sci. USA 87: 7235-7239 (1990); Rettig et al., Canc. Res. 53: 3327-3335 (1993); Garin-Chesa et al., Am. J. Pathol. 142: 557-567.

The results are shown in figure 5. Breast, colorectal, pancreas and lung carcinomas showed strong expression of FAPα and not CD26 was found (see figures 5A and 5B). Five FAPα+ sarcomas, including malignant fibrous histocytoma (figures 5C and 5D), were tested, and there was no expression of CD26. Examination of reactive fibroblasts of healing dermal wounds (figures 5E, 5F), showed abundant expression of both FAPα and CD26. The three renal carcinomas tested (figures 5G, 5H), showed expression of CD26 in malignant epithelium. FAPα was absent from malignant epithelial cells, and showed low expression in the stroma of these carcinomas.

The foregoing examples describe an isolated nucleic acid molecule which codes for fibroblast activating protein alpha ("FAPα"). The expression product of the sequence is a protein which, on SDS-PAGE, shows a molecular weight of about 75 kd. Deduced amino acid sequence, as provided in SEQ ID NO: 1, for one form of the molecule, yields a molecular weight of about 88 kd. It is to be understood that, as described, FAPα may be glycosylated, with the type and amount of glycosylation varying, depending upon the type of cell expressing the molecule. The experiment described herein shows this.

The invention also comprehends the production of expression vectors useful in producing the FAPα molecule. In their broadest aspect, these vectors comprise a FAPα coding sequence, operably linked to a promoter. Additional elements

may be a part of the expression vector, such as genes which confer antibiotic resistance, amplifiable genes, and so forth.

The coding sequences and vectors may also be used to prepare cell lines, wherein the coding sequence or expression vector is used to transfect or to transform a recipient host. The type of cell used may be prokaryotic, such as E. coli, or eukaryotes, such as yeast, CHO, COS, or other cell types.

The identification of nucleic acid molecules such as that set forth in SEQ ID NO: 1 also enables the artisan to identify and to isolate those nucleic acid molecules which hybridize to it under stringent conditions. "Stringent condition" as used herein, refers to those parameters set forth supra, whereby both murine and hamster sequences were also identified. It will be recognized by the skilled artisan that these conditions afford a degree of stringency which can be achieved using parameters which vary from those recited. Such variance is apprehended by the expression "stringent conditions".

The ability of nucleic acid molecules to hybridize to complementary molecules also enables the artisan to identify cells which express FAPα, via the use of a nucleic acid hybridization assay. One may use the sequences described in the invention to hybridize to complementary sequences, and thus identify them. In this way, one can target mRNA, e.g., which is present in any cell expressing the FAPα molecule.

It is of course understood that the nucleic acid molecules of the invention are also useful in the production of recombinant FAPα. The recombinant protein may be used, e.g., as a source of an immunogen for generation of antibodies akin to known mAb F19, and with the same uses. Similarly, the recombinant protein, and/or cells which express the molecule on its surface, may be used in assays to determine antagonists, agonists, or other molecules which interact with the FAPα molecule. Such molecules may be, but are not necessarily limited to, substrates, inhibiting molecules, antibodies, and so forth. This last feature of the invention should be considered in light of the observed structural resemblances to membrane bound enzymes. This type of molecule

is associated with certain properties which need not be described in detail here. It will suffice to say that inhibition or potentiation of these properties as associated with FAPα is a feature of this invention. For example, one may identify substrates or the substrate for the FAPα molecule, via the use of recombinant cells or recombinant FAPα per se. The substrates can be modified to improve their effect, to lessen their effect, or simply to label them with detectable signals so that they can be used, e.g., to identify cells which express FAPα. Study of the interaction of substrate and FAPα, as well as that between FAPα and any molecule whatsoever, can be used to develop and/or to identify agonists and antagonists of the FAPα molecule.

Other aspects of the invention will be clear to the skilled artisan, and need not be set forth here.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

(1) GENERAL INFORMATION:

(i) APPLICANTS: Rettig, Wolfgang J.; Scanlan, Matthew J.; Garin-Chesa, Pilar; Old, Lloyd J.

(ii) TITLE OF INVENTION: ISOLATED NUCLEIC ACID MOLECULE CODING FOR FIBROBLAST ACTIVATION PROTEIN α AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Felfe & Lynch
(B) STREET: 805 Third Avenue
(C) CITY: New York City
(D) STATE: New York
(E) COUNTRY: USA
(F) ZIP: 10022

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/230,491
(B) FILING DATE: 20-APRIL-1994
(C) CLASSIFICATION: 435

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Hanson, Norman D.
(B) REGISTRATION NUMBER: 30,946
(C) REFERENCE/DOCKET NUMBER: LUD 330

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 688-9200
(B) TELEFAX: (212) 838-3884

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2812 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AAGAACGCCC CCAAAATCTG TTTCTAATTT TACAGAAATC TTTTGAAACT TGGCACGGTA 60
TTCAAAAGTC CGTGGAAAGA AAAAAACCTT GTCCTGGCTT CAGCTTCCAA CTACAAAGAC 120
AGACTTGGTC CTTTTCAACG GTTTTCACAG ATCCAGTGAC CCACGCTCTG AAGACAGAAT 180
TAGCTAACTT TCAAAAACAT CTGGAAAAAT GAAGACTTGG GTAAAAATCG TATTTGGAGT 240
TGCCACCTCT GCTGTGCTTG CCTTATTGGT GATGTGCATT GTCTTACGCC CTTCAAGAGT 300
TCATAACTCT GAAGAAAATA CAATGAGAGC ACTCACACTG AAGGATATTT TAAATGGAAC 360
ATTTTCTTAT AAAACATTTT TTCCAAACTG GATTTCAGGA CAAGAATATC TTCATCAATC 420
TGGAGATAAG AATATAGTAC TTTATAATAT TGAAACAGGA CAATCATATA CCATTTTGAG 480
TAATAGAACG ATGAAAAGTG TGAATGCTTC AAATTACGGC TTATCACCTG ATCGGCAATT 540
TGTATATCTA GAAAGTGATT ATTCAAAGCT TTGGAGATAC TCTTACACAG CAACATATTA 600
CATCTATGAC CTTAGCAATG GAGAATTTGT AAGAGGAAAT GAGCTTCCTC GTCCAATTCA 660
GTATTTATGC TGGTCGCCTG TTGGGAGTAA ATTAGCATAT GTCTATCAAA ACAATATCTA 720
TTTGAAACAA AGACCAGGAG ATCCACCTTT TCAAATAACA TTTAATGGAA GAGAAAATAA 780
AATATTTAAT GGAATCCCAG ACTGGGTTTA TGAAGAGGAA ATGCTTCCTA CAAAATATGC 840
TCTCTGGTGG TCTCCTAATG GAAAATTTTT GGCATATGCG GAATTTAATG ATAAGGATAT 900
ACCAGTTATT GCCTATTCCT ATTATGGCGA TGAACAATAT CCTAGAACAA TAAATATTCC 960
ATACCCAAAG GCTGGAGCTA AGAATCCCGT TGTTCGGATA TTTATTATCG ATACCACTTA 1020
CCCTGCGTAT GTAGGTCCCC AGGAAGTGCC TGTTCCAGCA ATGATAGCCT CAAGTGATTA 1080
TTATTTCAGT TGGCTCACGT GGGTTACTGA TGAACGAGTA TGTTTGCAGT GGCTAAAAAG 1140
AGTCCAGAAT GTTTCGGTCC TGTCTATATG TGACTTCAGG GAAGACTGGC AGACATGGGA 1200
TTGTCCAAAG ACCCAGGAGC ATATAGAAGA AAGCAGAACT GGATGGGCTG GTGGATTCTT 1260
TGTTTCAAGA CCAGTTTTCA GCTATGATGC CATTTCGTAC TACAAAATAT TTAGTGACAA 1320
GGATGGCTAC AAACATATTC ACTATATCAA AGACACTGTG GAAAATGCTA TTCAAATTAC 1380
AAGTGGCAAG TGGGAGGCCA TAAATATATT CAGAGTAACA CAGGATTCAC TGTTTTATTC 1440
TAGCAATGAA TTTGAAGAAT ACCCTGGAAG AAGAAACATC TACAGAATTA GCATTGGAAG 1500
CTATCCTCCA AGCAAGAAGT GTGTTACTTG CCATCTAAGG AAAGAAAGGT GCCAATATTA 1560
CACAGCAAGT TTCAGCGACT ACGCCAAGTA CTATGCACTT GTCTGCTACG GCCCAGGCAT 1620
CCCCATTTCC ACCCTTCATG ATGGACGCAC TGATCAAGAA ATTAAAATCC TGGAAGAAAA 1680
CAAGGAATTG GAAAATGCTT TGAAAAATAT CCAGCTGCCT AAAGAGGAAA TTAAGAAACT 1740

TGAAGTAGAT GAAATTACTT TATGGTACAA GATGATTCTT CCTCCTCAAT TTGACAGATC 1800
AAAGAAGTAT CCCTTGCTAA TTCAAGTGTA TGGTGGTCCC TGCAGTCAGA GTGTAAGGTC 1860
TGTATTTGCT GTTAATTGGA TATCTTATCT TGCAAGTAAG GAAGGGATGG TCATTGCCTT 1920
GGTGGATGGT CGAGGAACAG CTTTCCAAGG TGACAAACTC CTCTATGCAG TGTATCGAAA 1980
GCTGGGTGTT TATGAAGTTG AAGACCAGAT TACAGCTGTC AGAAAATTCA TAGAAATGGG 2040
TTTCATTGAT GAAAAAAGAA TAGCCATATG GGGCTGGTCC TATGAGATAC GTTTCATCAC 2100
TGGCCCTTGC ATCTGGAACT GGTCTTTTCA AATGTGGTAT AGCAGTGGCT CCAGTCTCCA 2160
GCTGGGAATA TTACGCGTCT GTCTACACAG AGAGATTCAT GGGTCTCCCA ACAAAGATGA 2220
TAATCTTGAG CACTATAAGA ATTCAACTGT GATGGCAAGA GCAGAATATT TCAGAAATGT 2280
AGACTATCTT CTCATCCACG GAACAGCAGA TGATAATGTG CACTTTCAAA ACTCAGCACA 2340
GATTGCTAAA GCTCTGGTTA ATGCACAAGT GGATTTCCAG GCAATGTGGT ACTCTGACCA 2400
GAACCACGGC TTATCCGGCC TGTCCACGAA CCACTTATAC ACCCACATGA CCCACTTCCT 2460
AAAGCAGTGT TTCTCTTTGT CAGACTAAAA ACGATGCAGA TGCAAGCCTG TATCAGAATC 2520
TGAAAACCTT ATATAAACCC CTCAGACAGT TTGCTTATTT TATTTTTTAT GTTGTAAAAT 2580
GCTAGTATAA ACAAACAAAT TAATGTTGTT CTAAAGGCTG TTAAAAAAAA GATGAGGACT 2640
CAGAAGTTCA AGCTAAATAT TGTTTACATT TTCTGGTACT CTGTGAAAGA AGAGAAAAGG 2700
GAGTCATGCA TTTTGCTTTG GACACAGTGT TTTATCACCT GTTCATTTGA AGAAAAATAA 2760
TAAAGTCAGA AGTTCAAAAA AAAAAAAAAA AAAAAAAAAA GCGGCCGCTC GA 2812

We claim:

1. Isolated nucleic acid molecule which codes for mammalian FAPα having a molecular weight of about 88 kilodaltons based upon its deduced amino acid sequence.

2. The isolated nucleic acid molecule of claim 1, wherein said FAPα consists of the amino acid sequence set forth in SEQ ID NO: 1.

3. The isolated nucleic acid molecule of claim 1, consisting of the nucleotide sequence of SEQ ID NO: 1.

4. Isolated nucleic acid molecule which hybridizes to the nucleotide sequence of SEQ ID NO: 1, under stringent conditions.

5. Expression vector comprising the isolated nucleic acid molecule of claim 1, operably linked to a promoter.

6. Cell line transformed or transfected by the isolated nucleic acid molecule of claim 1.

7. Cell line transformed or transfected by the expression vector of claim 5.

8. Method for determining expression of FAPα in a cell comprising contacting said cell with the isolated nucleic acid molecule of claim 1 and determining hybridization of said isolated nucleic acid molecule to a complementary sequence in said cell as a determination of expression of FAPα.

FIG. 1

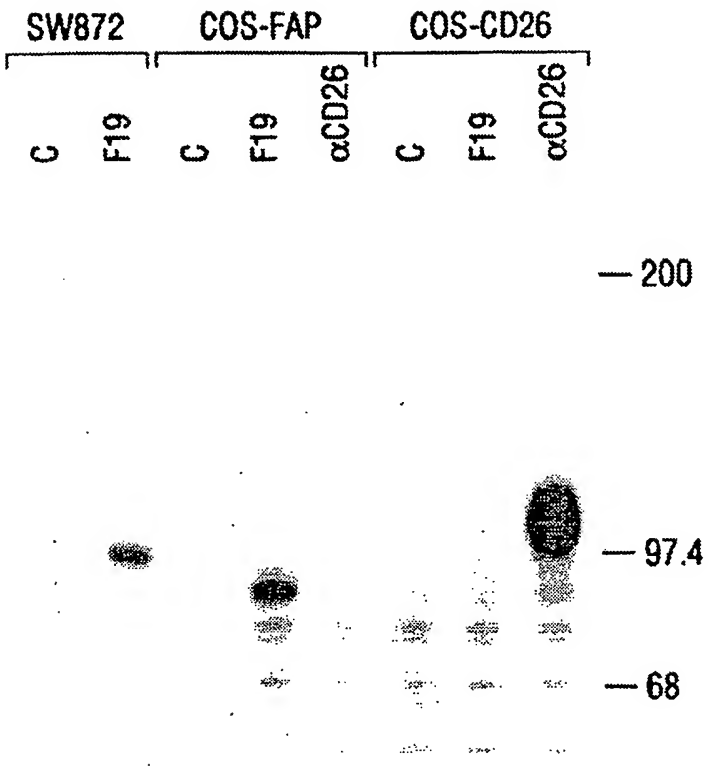


FIG. 2A

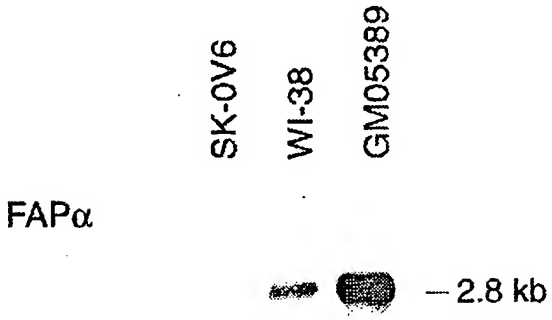

SK-0V6
WI-38
GM05389
FAPα
2.8 kb

FIG. 2B

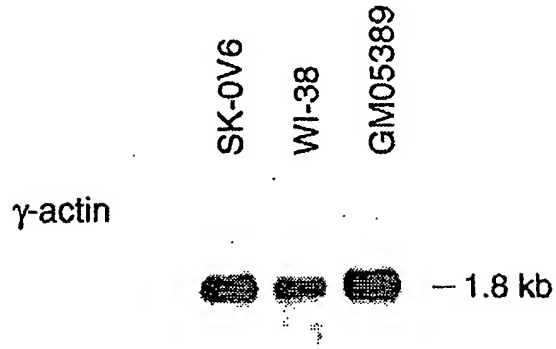
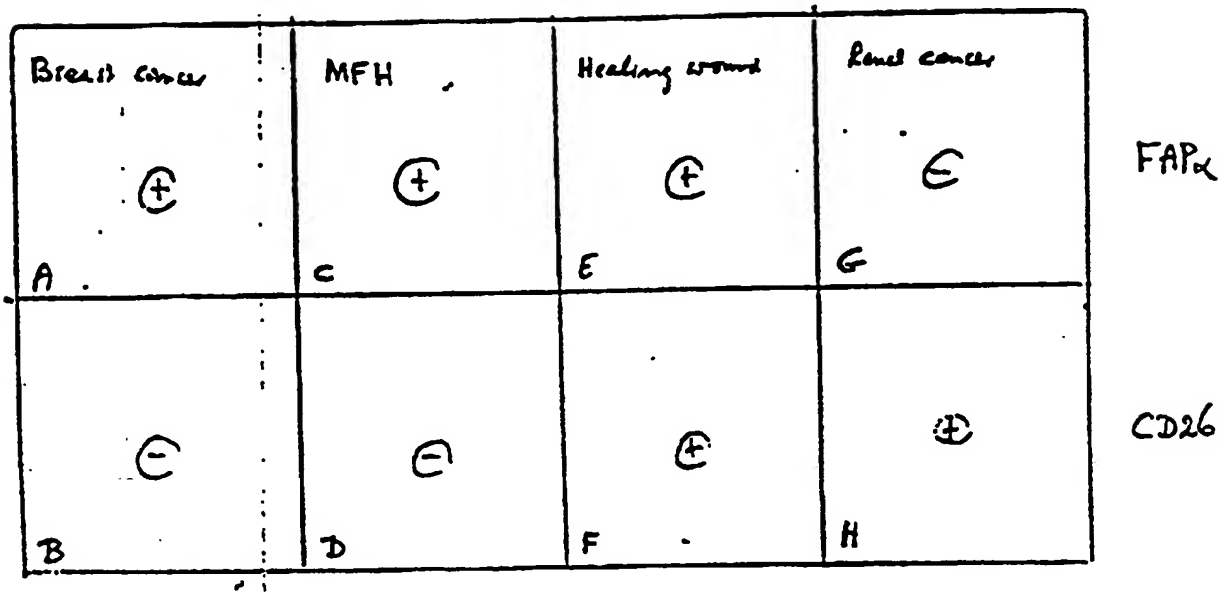

SK-0V6
WI-38
GM05389
γ-actin
1.8 kb

FIG. 3

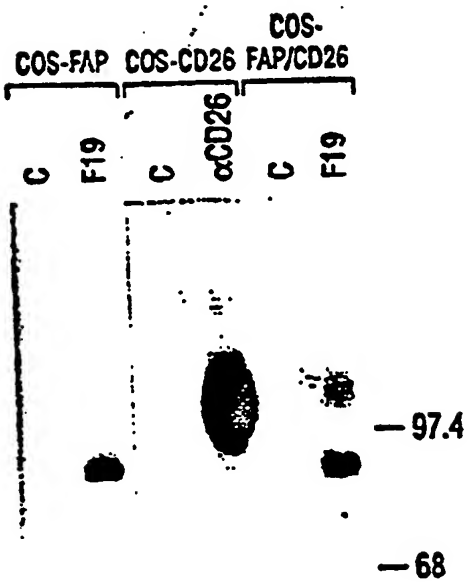
```
FAP     1 MKTWVKIVFGV*ATSAVLALLVMCIVLRPSRVHNSEENTMRALTLKDILN   49
CD26    1 ---PW-VLL-LLGAA-LVTIITVPV--LNKGTDDATADSRKTY--T-Y-K   50

FAP    50 GTFSYKTFFPNWISGQEYLHQSADNNIVLYNIETGQSYTILSNRTMKSV*   98
CD26   51 N-YRL-LYSLR---DH---YKQ*E---LVF-A-Y-N-SVF-E-S-FDEFG   99

FAP    99 *NASNYGLSPDRQFVYLESDYSKLWRYSYTATYYIYDLSNGEFVRGNELP  147
CD26  100 HSIND-SI---G--IL--YN-V-Q--H----S-D----NKRQLITEERI-  149
                                                      fap-1
FAP   148 RPIQYLCWSPVGSKLAYVYQNNIYLKQRPGDPPFQITFNGRENKIFNGIP  197
CD26  150 NNT-WVT-----H-----WN-D--V-IE-NL-SYR--WT-K-DI-Y---T  199
                                fap-2
FAP   198 DWVYEEEMLPTKYALWWSPNGKFLAYAEFNDKDIPVIAYSYYGDE**QYP  245
CD26  200 -------VFSAYS--------T-----Q---TEV-L-E--F-S--SL---  249

FAP   246 RTINIPYPKAGAKNPVVRIFIIDT***TYPAYVGPQEVPVPAMIASSDYY  292
CD26  250 K-VRV-------V--T-KF-VVN-DSLSSVTNATSIQITA--SMLIG-H-  299

FAP   293 FSWLTWVTDERVCLQWLKRVQNVSVLSICDFREDWQTWDCPKTQEHIEES  342
CD26  300 LCDV--A-Q--IS----R-I--Y--MD---YD-SSGR-N-LVARQ---M-  349

FAP   343 RTGWAGGFFVSRPVFSYDAISYYKIFSDKDGYKHIHYIKDTVENAIQITS  392
CD26  350 T---V-R-RP-E-H-TL-GN-F---I-NEE--R--C-FQIDKKDCTF--K  399

FAP   393 GKWEAINIFRVTQDSLFYSSNEFEEYPGRRNIYRISIGSYPPSKKCVTCH  442
CD26  400 -T--V-G-EAL-S-Y-Y-I---YKGM--G--L-K-QLSD-T*KVT-LS-E  448

FAP   443 LRKERCQYYTASFSDYAKYYALVCYGPGIPISTLHDGRTDQEIKILEENK  492
CD26  449 -NP------SV---KE----Q-R-S---L-LY---SSVN-KGLRV--D-S  498
                          fap-3
FAP   493 ELENALKNIQLPKEEIKKLEVDEITLWYKMILPPQFDRSKKYPLLIQVYG  542
CD26  499 A-DKM-Q-V-M-SKKLDFIILN-TKF--Q-----H--K-------LD--A  548

FAP   543 GPCSQSVRSVFAVNWISYLASKEGMVIALVDGRGTAFQGDKLLYAVYRKL  592
CD26  549 -----KADT--RL--AT----T-NIIV-SF----SGY----IMH-IN-R-  598

FAP   593 GVYEVEDQITAVRKFIEMGFIDEKRIAIWGWSYEIRFITGPCIWNWSFQM  642
CD26  599 -TF------E-A-Q-SK---V-N----------GGYVTSMVLGSGSVGFK  648

FAP   643 WYSSGSSLQLGILRVCLHRE*IHGSPNKDDNLEHYKNSTVMARAEYFRNV  691
CD26  649 CGIAVAPVSRWEYYDSVYT-RYM-L-TPE---D--R-----S---N-KQ-  698

FAP   692 DYLLIHGTADDNVHFQNSAQIAKALVNAQVDFQAMWYSDQNHGLSGLSTN  741
CD26  699 E---------------Q----S----DVG--------T-ED--IASSTAH  748

FAP   742 *HLYTHMTHFLKQCFSLSD
CD26  749 Q-I----S--I------P
```

Fig. 5



Immunohistochemistry (see Kodachromes)

Fig. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04860

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/00, 15/09, 15/12
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 69.1, 172.3, 240.2, 252.3, 320.1; 536/23.5, 24.3, 24.31, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155,351,357,358) search terms: FAP, fibroblast activation protein, antigen, CD26

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ---- A	CANCER RESEARCH, Volume 53, issued 15 July 1993, W.J. Rettig et al, "Regulation and Heteromeric Structure of the Fibroblast Activation Protein in Normal and Transformed Cells of Mesenchymal and Neuroectodermal Origin", pages 3327-3335, see entire document.	1-7 ----- 8
Y ---- A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, Volume 85, issued May 1988, W.J. Rettig et al, "Cell-surface glycoproteins of human sarcomas: Differential expression in normal and malignant tissues and cultured cells", pages 3110-3114, see entire document.	1-7 ---- 8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
21 JUNE 1995

Date of mailing of the international search report
10 JUL 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
MARIANNE PORTA ALLEN
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04860

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, Volume 84, issued December 1987, A. Aruffo et al, "Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system", pages 8573-8577, see entire document.	1-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/04860

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 69.1, 172.3, 240.2, 252.3, 320.1; 536/23.5, 24.3, 24.31, 24.33